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Effects of Silver on Ethylene Synthesis and Action in Cut Carnations

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Abstract. Silver, applied as silverthiosulphate, completely blocked the ethylene surge preceding the wilting of the petals. As a consequence, vase life was extended by nearly 100%. In addition, a pretreatment with silverthiosulphate caused the flowers to become insensitive to an ethylene treatment.

Key words: *Dianthus* – Ethylene action – Ethylene synthesis – Flower longevity – Silver.

Introduction

It has been shown recently that the silver ion acts as a potent anti-ethylene agent in various plants and thereby improves longevity (Beyer, 1976a). The longevity of cut carnations can also be increased by pretreatment with silver salts (Halevy and Kofranek, 1977). Due to the relative immobility of the silver ion, a basal treatment of the stem was less effective than a direct spray treatment of the flower. The formation of black spots on the petals limits the practical use of this latter pretreatment. The immobility of the silver ion inside the transport system of the plant might be due to the participation of the silver ion in the cation-exchange processes at the negatively charged sites of the walls of the xylem vessels. The low mobility of silver can be increased by chelating the metal to an anionic complex such as silverthiosulphate. A detailed report as to how the silverthiosulphate anionic complex is transported at a speed of 2 m h^{-1} inside the stems of cut carnations has been published (Veen and Van de Geijn, 1978). The anti-ethylene action of silver is preserved in this complex, as shown by a significant improvement in the longevity of carnation flowers in the presence or absence of ethephon, an ethylene-releasing compound. From these data it is obvious that silver blocks the action of ethylene.

This paper deals with the question of whether the silver ion also antagonizes the endogenous production of ethylene. Beyer (1976b) suggests that the effect of silver ion on the sex expression of cucumber plants (silvernitrate caused staminate flowers to develop) "is apparently not related to endogenous ethylene production, since preliminary data suggest that ethylene production is unaffected by nontoxic concentrations of silvernitrate and stimulated by higher toxic levels." Also, Beutelmann and Kende (1977) describe the antiethylene effect of the silver ion and found that "although silver inhibited rolling-up of the rib segment of *Ipomoea* flowers, it did not affect either spontaneous and ethylene-induced ethylene generation." As Beyer and Beutelmann and Kende used the extremely low mobile Ag^+ ion (applied as silvernitrate), it is likely that while the silver (Ag^+) ion reached the site of ethylene action, it did not reach the site of production. This suggestion is supported by the observation of Saltveit et al. (1978) that Ag^+ applied as $AgNO_3$ inhibits ethylene synthesis in ripening fruit (bananas, tomatoes and apples) if the silver salt is applied to the tissue by means of an infiltration technique.

The aim of the experiments presented in this paper is to investigate the possible inhibitory effect of silver, applied as silverthiosulphate, on the ethylene synthesis of cut carnations.

Materials and Methods

Carnation flowers (*Dianthus caryophyllus* L., cv. White Sim) were harvested in a commercial nursery, trimmed to an uniform length of 45 cm, and kept out of water overnight at 4° C. Stems were subsequently immersed for 24 h in a solution of silverthiosulphate ($Ag(S_2O_3)_2^{3-}$), prepared by mixing equal volumes of silvernitrate ($AgNO_3$) and sodiumthiosulphate ($Na_2S_2O_3 \cdot 5\,H_2O$) of various concentrations but always in the ratio 1 : 8, respectively. After the pretreatment the stems were placed in de-ionized water or in an ethephon solution of 50 mg l^{-1}.

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The experiments were carried out in a greenhouse compartment under natural daylight conditions. The temperature varied between 16° C and 24° C, and the relative humidity between 60 and 70%. The longevity was determined as the mean number of days after harvest until initial wilting or rolling-in of the petals (Halevy and Kofranek, 1977).

The ethylene production of flowers with a stem length of 10 cm was measured by placing 5 flowers in a desiccator with an air volume of about 2 l. The atmosphere was sampled in a standard procedure after 3 h and ethylene was determined using a flame ionization gas chromatograph (Packard, Model 428) with an activated alumina oxide column and an oven temperature of 40° C. Ethylene was identified by retention time and co-chromatography with ethylene.

The ethylene production of flower parts viz. sepals, petals, bracts, receptacle, ovary and styles, was measured by placing these parts into small vials which were sealed with a rubber septum. A 1.0 ml gas sample was withdrawn by syringe after 1 h.

Results

The relationship among the applied concentration of silverthiosulphate, the ethylene production, and the longevity was investigated. In a previous paper (Veen and Van de Geijn, 1978) silverthiosulphate was applied in a final concentration of 2.0 mM. Some harmful effects, i.e. brownish spots on the leaves caused by the toxic action of silver, were observed. In the experiments presented in this paper, 10-, 20-, and 100-fold lower concentrations were tested. The ethylene produced by flowers pretreated with these solutions together with the longevity data are presented in Fig. 1. Only small amounts of ethylene were produced by intact flowers during the first five days after harvest. On the 6th day a significant increase in ethylene production occurred in the controls which reached a maximum value on the 8th day. Rolling-in of the petals occurred after 8 days, coinciding with a decrease in the ethylene production. Carnation flowers pretreated by a stem-base treatment with 0.1, 0.2, and 2.0 mM silverthiosulphate for 24 h, did not show the ethylene surge after 6–8 days, although the basal ethylene production in intact flowers of approximately 2–5 nl h^{-1} $flower^{-1}$ was not influenced by this pretreatment. The flowers pretreated with 0.1, 0.2, and 2.0 mM silverthiosulphate did not show any sign of wilting within the experimental period. The data in Fig. 1 further show that while a concentration of 0.02 mM silverthiosulphate caused some delay in wilting, it did not prevent the ethylene surge.

The increase in ethylene production in the silverthiosulphate-treated flowers after 12 days (see Fig. 1) is not well understood. It may be that this increase is related to the tissue damage which was caused by an excessive transfer of the flowers to and from the desiccators. The evidence for this is provided by a

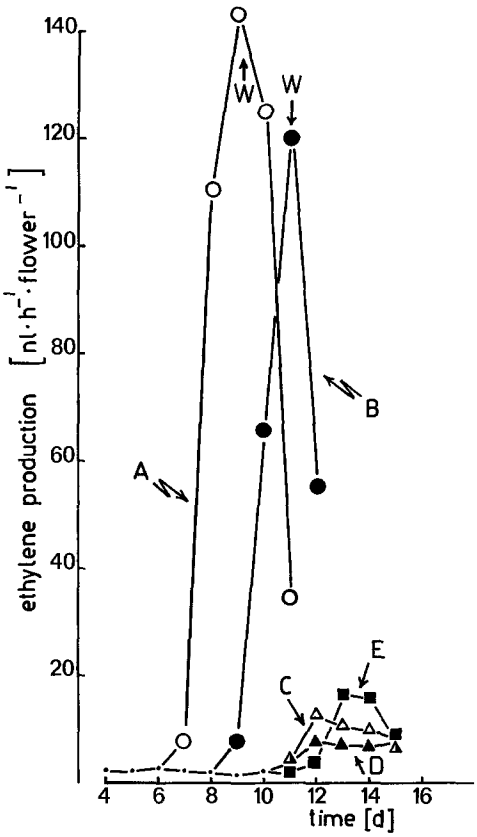

Fig. 1. Ethylene production by intact carnation flowers pretreated for 24 h with the following silverthiosulphate solutions, A (○—○): 0.0 mM (controls); B (●—●): 0.02 mM; C (△—△): 0.1 mM; D (▲—▲): 0.2 mM; E (■—■): 2.0 mM. W indicates time of initial wilting

Table 1. Inhibition of ethylene production in carnations by floral spray treatment with silvernitrate (0.2 mM) and with silverthiosulphate (0.2 mM). Stem base treatment with silverthiosulphate for 24 h (0.2 mM). All flowers were placed on water after pretreatments

Days in water	Production ethylene nl h^{-1} $flower^{-1}$			
	Control	Flower spray		Stem base treatment
	+Tw 0.1%	$AgNO_3$ +Tw 0.1%	$Ag(S_2O_3)_2^{3-}$ +Tw 0.1%	$Ag(S_2O_3)_2^{3-}$
3	2.3	2.5	2.8	2.8
5	4.6	1.5	5.3	4.3
6	75.4	–	4.2	4.0
7	382.0	56.2	91.2	4.4
8	169.6	112.0	108.1	6.8
9	71.5	–	96.5	11.4
10	9.9	28.6	21.9	12.1
11	4.2	3.0	4.7	2.9
13	9.4	3.4	5.5	2.8
Flowers wilt after (days)	8	11	11	>13

brownish discoloration of the petal rims observed after about 13 days.

Floral spray treatments with silver nitrate significantly delayed senescence of carnation flowers (Halevy and Kofranek, 1977). The ethylene production after such a pretreatment was, therefore, also studied. A test run was also made with a floral spray treatment with silverthiosulphate (Table 1). A flower spray with $AgNO_3$ or $Ag(S_2O_3)_2^{3-}$ delayed senescence but did not block the ethylene production. The data in Table 1 indicated further that silver can only block the ethylene production if it is fed to the flower via the transpiration stream.

Nichols (1977) showed that in the unpollinated carnation flower the style and the petals, independently of each other, can show a surge in ethylene. Thus, petal senescence is not necessarily dependent upon stylar ethylene. Nichols further showed that the bases of petals from senescing flowers evolved ethylene faster than the upper parts. As silver accumulates inside the receptacle (Veen and Van de Geijn, 1978), which includes the basal end of the petals, the ethylene production of the receptacle tissue was studied (Table 2). From these data it is obvious that the receptacle is also a potential source of high ethylene production. It has been further shown that the production of ethylene by the receptacle tissue is fully blocked by a pretreatment with silverthiosulphate.

It is well-known that the total production of ethylene by the flower can be promoted – due to the autocatalytic action – by small amounts of exogenously applied ethylene. An estimation of the amount of this self-generated ethylene can be made by a comparison of ethylene production in ethephon-treated flowers, with or without a pretreatment with silverthiosulphate. After being harvested, flowers were kept dry and cool during the first day, thereafter they were pretreated for 24 h with silverthiosulphate (0.2 mM). Controls were placed for the same period in de-ionized water. Subsequently, the treated flowers and the controls were transferred to an ethephon solution of 50 mg l^{-1}. On the 4th day, control flowers wilted and showed a maximal ethylene production of about 1400 nl ethylene h^{-1} $flower^{-1}$ (see Fig. 2). Flowers pretreated with silverthiosulphate fully escaped the effect of the ethephon treatment and although they produced ethylene in great quantities (650 nl h^{-1} $flower^{-1}$) after 6 days, they did not wilt. The difference between ethylene production in control flowers (not pretreated with silverthiosulphate but treated with ethephon) and the flowers treated sequentially with silverthiosulphate and ethephon is undoubtedly due to self-generated ethylene. Apparently, silver pretreatment antagonizes this autokatalytic production of ethylene.

Table 2. Mean ethylene production from various flower parts after different time intervals

	Ethylene production nl h^{-1} flower part^{-1} Time in days[a]					
	1	3	5	7	9	11
bracts	0.06	0.12	0.10	0.21	0.08	0.19
sepals	0.12	0.18	0.18	0.83	0.23	0.28
petals	1.38	2.21	2.94	54.56	97.34	3.28
receptacle	0.28	0.45	0.56	32.72	6.08	5.64
ovary	0.37	0.76	0.42	11.35	3.86	3.03
styles	0.29	0.31	0.83	6.15	2.14	0.28

[a] Flowers wilt after 8 days

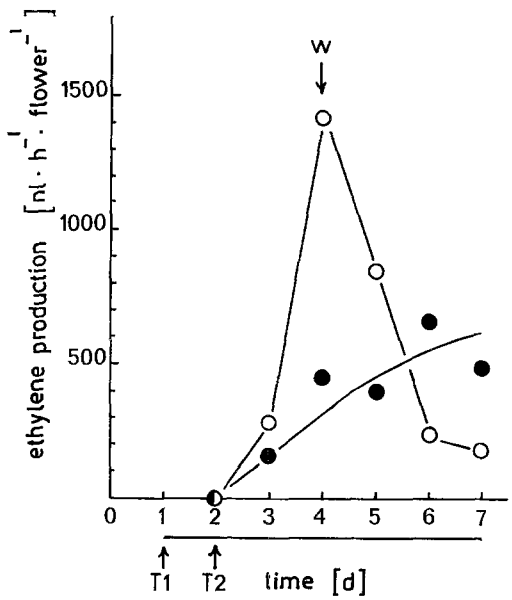

Fig. 2. Ethylene production by intact carnation flowers pretreated with silverthiosulphate for 24 h at the indicated time T 1 and subsequently placed in an ethephon solution of 50 mg l^{-1} at the indicated time T 2 (●—●). Control flowers were placed on water at time T 1 and placed on ethephon of the same concentration at time T 2 (o—o). W indicates time of initial wilting

Discussion

Mayak et al. (1977) summarize the sequence of senescence phenomena in the carnation flower. There is a low steady-state level of ethylene which may be viewed as an equilibrium between a low and a fairly constant production rate and a diffusion from the flower (phase 1). Subsequently an increase in the intercellular ethylene concentration occurs by some unknown cause (phase 2). This ethylene production leads to an increase in tonoplast permeability. A decline in water uptake by the flower coincides with or probably closely follows this increased tonoplast

permeability. Petal wilting is visible 2 h after this decline.

The use of silver salts to antagonize ethylene action can be a valuable tool to elucidate the primary action of this plant hormone. The silver ion does not interfere with phase 1, as can be concluded from our data in Table 1 and Figure 1. Our experiments show that pretreated flowers show an ethylene production which is of the same level as that of the control flowers. Phase 2 is blocked by silver in a very effective way. According to Beyer it cannot be concluded that silver scavenges the ethylene, it is more likely that silver interferes with some other processes, e.g. enzymatic reactions involved in ethylene biosynthesis. If this is so, the biosynthetic pathway of ethylene during the surge which takes place after 7–8 days (phase 2) is different from the one during phase 1.

In the experiments, presented in this paper, ethephon was used as an ethylene-releasing compound. Nichols (personal communication) found that the silver complex prevented such visible phenomena as those associated with ethylene damage (by a gaseous ethylene treatment of 1 vpm for 24 h) to carnation, viz. irreversible petal wilting, swelling of the ovary, and greening of the ovary wall. From these data it is obvious that silver blocks the action of ethylene. Moreover silver protects the flower from the wilting caused by ethephon which induces the dramatic surge in ethylene production; a process completely blocked by silverthiosulphate pretreatment (see Fig. 2).

According to Beyer (1976a) the effect of Ag^+ could be explained on the basis that Ag^+ substitutes for Cu^+. It is proposed that Cu^+ is the metal involved in enzymic reactions related to the biosynthesis or the action of ethylene. To quote Beyer, "the similarity in size of Ag^+ and Cu^+, the same oxidation state, and the ability of both metals to form complexes with ethylene lend credence to this idea." Beyer (1977) furthermore suggests that Ag^+ inhibits $^{14}C_2H_4$ incorporation in carnation flowers without affecting its oxidation. This ethylene incorporation occurs especially in the reproductive and receptacle tissue and at a time just before the surge in ethylene production. The inhibitory effect of silver on the ethylene surge after 7–8 days may be related to this blockage of ethylene incorporation. This may also explain why the tissue becomes ethylene-insensitive after a pretreatment with silver. The significant role of the receptacle tissue in flower senescence is further strengthened by the observation that this tissue can produce ethylene in high quantities (see Table 2).

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